[0148] Currently the improvement of tissue culture practices arises via hypothesis, evaluation and adoption. Hypotheses arise from observation of size, shape, weight, etc. and physiological measurement of ion or sugar content (Figure 6, box 1). These observations are limited in scope and this limits the improvements that can be made to the tissue culture process. Gene expression is closely linked to metabolic condition, thus the observation of which genes are induced or repressed under a given growth condition, naturally, on the tree, or in a culture vessel, provides insight into the metabolic state of the embryo. This information can be used to create new hypotheses that can be evaluated by modifying tissue culture.

[0149] To this end, mRNA levels of two cDNAs (LPZ-202 and LPZ-216), similar to "Late Embryogenesis Abundant" (LEA) proteins, identified in other plants, were monitored. These genes are induced by the plant hormone ABA. Two peaks of mRNA were observed in these clones rather than the typical single peak in most plants. (See Figure 4 for clone LPZ-216; clone LPZ-202 is similar but data is not shown.) It was subsequently confirmed that two peaks in ABA activity are observed during development and that these correspond in timing to the elevation in mRNA for LPZ-202 and LPZ-216. Thus mRNA abundance profiles are providing insight into embryo physiology. (See Figure 7) The effect of giving two pulses of ABA to our somatic embryos is assessed; a tissue culture modification that we might not have considered as important had the gene expression data been unavailable. Internal data shows fluctuations in the abundance of mRNA for cDNAs listed in this collection (data not shown.)

Zygotic and Somatic Loblolly Pine Embryos

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[0150] Loblolly pine cones were collected weekly from a breeding orchard near Lake Charles, Louisiana, and shipped on ice for experimentation. Embryos were excised and evaluated for developmental stage (Pullman et al. 1994). Stage 9 embryos were separated by the week they were collected - 9.1 (week 1), 9.2 (week 2), etc. Staged zygotic embryos were sorted into vials partially immersed in liquid nitrogen and stored at -70°C. Somatic embryos for loblolly pine were initiated as described by Becwar et al. (1995) or with minor modifications. Somatic embryos were grown, selected, and staged as described by Pullman et al. (1994) and stored at -70°C.

cDNA Probe Preparation and Hybridization

[0151] 30 ng of purified Lea protein cDNA fragments was labeled with ^{32}P dCTP using the Ready-To-Go cDNA Random Labeling kit (Pharmacia). The labeled cDNAs were purified using NICK Column (Pharmacia) and heat denatured for hybridization. The RNA slot blot was pre-hybridized in hybridization buffer (0.5 M sodium-phosphate, pH 7.2, 5% SDS, and 10 mM EDTA) at 65°C for 2 hours in a hybridization oven (Model 400, Robbins Scientific, Sunnyvale, CA) and the hybridized in the same conditions with the cDNA probes. After hybridization, the membranes were washed at 65°C in 0.2x SSC and 0.1 % SDS. Each wash was 15 min. The membranes were then exposed to Image Plate.

[0152] The probes can be stripped from the RNA slot blot by pouring boiling 0.5% SDS onto the membrane twice and incubating without heating for 30 min. The stripped blot was then exposed to Image Plate for overnight to check the completeness of the de-probing before next round of hybridization.

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[0153] To ensure the equal loading of the each RNA sample, the same membranes were stripped and hybridized with a ^{32}P-dCTP labeled 26S ribosomal rDNA fragment. These results were used as controls to normalize the Lea protein gene expression levels.

[0154] As a means of evaluating the usefulness of these arrays, we followed the expression of three cDNAs that have strong sequence similarity to late embryo-abundant proteins, (Lea) proteins from cotton (Baker et al 1988). Lea proteins and mRNAs appear in embryos at a stage when ABA is high and the genes can be induced in vegetative tissue by application of ABA. The transcript level of Lea genes LPZ-202 and LPZ-216 showed two peaks, rising from stage 5 and returning to a base line about stage 9.2 then rising again around stage 9.5. (See Figure 4 for clone LPZ-216).

[0155] To confirm the fluctuation in lea transcript levels by Northern analysis. RNA was extracted from zygotic embryos at different stages of development. A pine 'dehydrin' cDNA from the North Carolina State University cDNA collection (http://www.cbc.med.umn.edu/ResearchProjects/Pine/DOE.pine/index.html) was used as probe for some experiments. Dehydrins are a class of lea protein, originally identified as water deficit inducible proteins. Since the expression of this class of protein is well characterized, in contrast to our lea genes, the dehydrin expression profile could act as a reference point. After probing with dehydrin, blots were stripped and probed with a 26S rDNA probe from Arabidopsis to check the loading of the original gel. The normalized expression pattern of dehydrin in the zygotic embryogenesis is illustrated in the top panel of Figure 4. The expression of the dehydrin gene was induced at stage 5 and reached a peak at stage 6. It declined at stage 7 - 8, just prior to